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Separation and purification of a suspension with magnetic microparticles

The invention relates to a device for the automatic separation of the solid and liquid phase of a suspension and for purifying magnetic microparticles loaded with organic, in particular molecular biological or biochemical substances, which device comprises a process area with mechanisms which move in a cyclic manner for transporting the magnetic microparticles in the x-direction. The invention also relates to sample and reagent containers for use in the device and a method for automatic separation and purification in the device.

The investigation and/or analysis of organic, in particular molecular biological substances by determining their chemical or physical properties with specifically developed methods is continuously increasing in importance. The use of an inert carrier in the form of microparticles is advantageous for the chemical analysis of molecular biological substances, for example blood or urine. If these microparticles consist of a magnetic or magnetisable material, contain a material such as this or are covered with such a material, the solid phase can be separated off from a suspension with a magnetic field and isolated by subsequent purifying or washing processes with a very high degree of cleanliness. Non-magnetic microparticles are sedimented, aspirated off or decanted, which makes comparatively complicated, long-lasting and/or frequent washing processes with at least one buffer solution necessary.

Loaded magnetic microparticles are separated off according to known methods, in particular, in that they are deposited by permanent magnets on the wall of a reaction vessel with the formation of a cluster and are fixed there during pipetting or decanting of the suspension liquid. During the removal of the suspension liquid, the magnetic field has to be maintained, which generally results in complicated methods and devices.

A method and a device for separating and washing magnetic solid particles which are arranged finely distributed in liquid samples is described in DE 3926462 A1.

The solid particles are loaded with organic substances as the precursor for a photometric or radiometric evaluation of patient samples in the case of immunoluminometric and immunoradiometric tests. The liquid samples are exposed in test tubes to a magnetic field generated by permanent magnets with the magnetic solid particles being attached to the inner wall of the test tubes. After a predetermined time, the residual liquid is aspirated off while maintaining the magnetic field. The separation and washing process is carried out fully automatically in a continuous process and a plurality of permanent magnets are arranged on a conveying section for the test tubes.

The separation method for depositing magnetic microparticles is improved according to EP 0806665 B1, in that according to one variant, the microparticles which are deposited on the vessel wall under the effect of a permanent magnet can be resuspended, after the rinsing water has been aspirated off, with fresh water or a fresh reagent, and this substantially increases the purification effect. After a certain dwell time, the microparticles are again attached under the magnetic effect of the wall of the reaction vessel and the rinsing water is aspirated off again. This process can be repeated many times.

US 6207463 B1 discloses a separation of magnetic microparticles from a suspension, in that a rod-shaped permanent magnet, which is covered by a protective layer except for the point, is dipped into the suspension with magnetic microparticles. The magnetic microparticles attach to the point of the transfer element and can be removed from the suspension solution when the rod is lifted, the cluster adheres to the rod and can be dipped into a new liquid phase. Thus pipetting or decanting can be omitted.

The inventor has set himself the object of providing a device and a method which allow a fully automatic process sequence and can be applied easily, rapidly, reliably and economically.

With reference to the device, the object is achieved according to the invention in that a first guide is arranged for supplying sample containers in the x-direction and

second guides are arranged for supplying reagent containers in the y-direction to the process area, wherein the second guides extend in the y-direction at an angle α of 30 to 150° to the x-direction, a carrier element, which can be moved back and forth in the x-direction, comprises carrier plates which can be lifted and lowered in the z-direction, individually and together, for magnetic or magnetisable transfer elements which are arranged in a matrix shape, the reagent containers can be positioned according to the grid of the transfer elements by introduction, taking place at an angle α, into the process area and can be rejected by ejection in the same direction into a waste collector.

Special and developing embodiments of the invention are the subject of dependent claims.

Transfer elements are preferably configured as permanent magnetic rods or as rod-shaped electromagnets.

The lowermost part of the transfer elements, which dip into the sample and reagent containers, is expediently covered with a membrane which can be lifted and lowered, can be deposited and taken off by a relative movement with respect to the transfer elements and is preferably tubular or beaker-shaped. The membrane can be omitted in the case of electromagnetically operating transfer elements.

According to the prior art, cyclic batches constantly run one-dimensionally in the x-direction. According to the invention, the cyclic batches run two-dimensionally, the sample containers in the x-direction and the reagent containers, in contrast, in the y-direction. As is conventional in the case of space coordinates, the two directions preferably have an angle α of 90°, and they also run at right angles with respect to the third, vertical, space coordinate z. The first guide for supplying the sample containers in the x-direction is given; it runs in the same direction as the back and forth movement of the carrier element. In special embodiments on the other hand, the y-direction for the two guides may vary in a relatively large angle range. The second guides expediently extend in parallel but they may also spread

out and/or, rising, form a slide. Closed reagent containers, however, at the latest directly before the process area, have a horizontal position, so they can be loaded with a reagent or a possible closure can be torn off or penetrated without risk of the reagent leaking.

The relative movement of a transfer element in the longitudinal direction thereof compared to the membrane preferably takes place by different lifting and lowering of the relevant carrier plates or guides with means which are known *per se*. Obviously, this relative movement could partially also take place by means of lifting and lowering the carrier block located below for the reagent containers, but this seems less advantageous.

The transportation of the magnetic microparticles in the x-direction preferably takes place, as mentioned, on tubular or beaker-shaped cavities of the membrane in the lowermost region of the transfer elements when the forward movement of the carrier plates takes place in the x-direction and the pulled-up membrane can be lowered into the immediately following reagent container. These membranes are expendable materials; they are guided to the inlet side of the process area, positioned during a lowering movement, placed on the rearmost transfer elements in the x-direction and entrained. It must be possible to position the membranes in the reagent containers, about halfway up the maximum lifting height of the transfer elements, with and without an introduced transfer element. For the continuous supply of membranes to the inlet side of the process area, a third guide is provided which, with respect to the x-direction, has an angle of preferably 60 to 120°. The membranes are designed such that they can be introduced into the sample and reagent containers; in other words they are the same, in particular, with respect to number and form of the cavities. The membranes are also designed, like the sample and reagent containers, preferably as injection mouldings or deep-drawn parts made of plastics material.

The containers and the membranes are substantially strip-shaped, stackable cassettes with a plurality of beaker-shaped cavities corresponding to the grid of the transfer elements in the carrier element.

With regard to the method for automatically separating the solid and liquid phase of a suspension and for purifying the solid phase, the object of the invention is achieved in that the forward movement of the carrier element in the x-direction takes place with the use of permanent magnetic rods as transfer elements with loaded, pulled-up membranes or with the use of rod-shaped electromagnets with the current switched on, and the backward movement counter to the x-direction takes place with the use of permanent magnetic rods as transfer elements without membranes or with the use of rod-shaped electromagnets with the current switched off. Special and developing embodiments of the method are the subject of dependent claims.

Firstly, the filled sample containers are preferably guided intermittently or continuously on the longitudinal side in the x-direction and the reagent containers with different or at most partially the same fillings are guided continuously in the y-direction at the end face to the process area. With each initiation of a new operating cycle, one membrane in each case is put over the rearmost transfer elements in the x-direction, configured as permanent magnetic rods, the latter are lowered into the sample container disposed at the process area and, after attachment of the magnetic microparticles to the membrane, the transfer elements with the membrane are raised from the suspension liquids. The carrier element is displaced forward in the x-direction by a grid unit, corresponding to the spacing between two reagent containers, the particle-free sample container is ejected into a waste container. The filled reagent containers are simultaneously introduced into the process area, the carrier element with the transfer elements is lowered into the reagent container, the transfer elements are pulled out of the membranes, the attached magnetic microparticles are resuspended and the suspension mixed. The transfer elements are returned by the spacing a counter to the x-direction, while the membranes remain in their position.

On each movement of the carrier plates in the x-direction, the membranes are entrained by one grid unit, the spacing a, and at the end of the process area, ejected into a waste container. The last reagent container in the x-direction,

ejected from the process area, is supplied for any further use which is known *per se*, for example chemical analysis.

In the case of transfer elements which are configured as rod-shaped electromagnets, without a membrane, the current is switched on for loading with microparticles and switched off for resuspension.

One working cycle preferably lasts 2 to 4 min. The duration of one working cycle is as low as possible for economic reasons, currently about 2 min can be achieved.

With a full working load, 6 to 10 reagent containers are simultaneously pushed into the process area. All the reagent containers preferably have different reagents, with pure water or an organic solution also being called a reagent. Obviously, however, sequences with individual or repeating reagents may be put together. Within the same reagent container, the cavities always have the same reagent. If not all the channels (48 in Fig. 17) are occupied by reagent containers, the frontmost channels in the x-direction remain empty. The rejection of the membranes and the further use of the frontmost reagent container takes place as if it were in the frontmost channel.

The microparticles used, as the name states, have dimensions from one to a plurality of micrometres and they may also have fractions of a micrometre and should then correctly be called nanoparticles. For the sake of simplicity, however, the term microparticles will be used for all particle sizes. The cavities of the sample and reagent containers generally have a volume of 1 to 3 ml.

The advantages of the invention can be summarised as follows.

- The device according to the invention can be operated fully automatically.
- The method with the working cycles allows all the cavities to be in action during the entire process.

- Numerous modules, generally six to ten, can operate simultaneously, which means maximum working productivity.
- The matrix arrangement allows an optimally dense arrangement, resulting in a further increase in productivity.
- The method allows continuous processing of samples.

The invention will be described in more detail with the aid of embodiments shown in the drawings, which are also the subject of dependent claims. In the drawings, schematically:

Fig. 1 shows a perspective view of the device,

Fig 2 shows a vertical section in the x-direction through the process area,

Fig. 3 shows a layout of the device at the beginning of the process,

Fig. 4 shows a vertical section in the x-direction through the process area with the positioned sample container,

Fig. 5 shows a subsequent process step according to Fig. 4, with the permanent magnetic rods dipped in the sample container, with membranes,

Fig. 6 shows a next process step according to Fig. 5 with a carrier element displaced in the x-direction,

Fig. 7 shows a layout of the device after filling of the first reagent container,

Fig. 8 shows a further process step according to Fig. 6 with the permanent magnetic rods pulled up and the membrane in the first reagent container,

Fig. 9 shows a further variant with a carrier element returned in the counter direction to the x-direction,

Fig. 10 shows a further method step according to Fig. 9 with the second membrane in place,

Fig. 11 shows a further method step according to Fig. 10 with lowered permanent magnetic rods,

Fig. 12 shows a further layout with an ejected first reagent container,

Fig. 13 shows a further layout of the device with the carrier element moved forward in the x-direction,

Fig. 14 shows a further method step according to Fig. 13 with the carrier element displaced in the x-direction,

Fig. 15 shows a further layout after a plurality of method steps with the first reagent container in the end position,

Fig. 16 shows a last layout with an ejected reagent container and membrane,

Fig. 17 shows a partially cut away perspective view of a carrier block,

Fig. 18 shows a horizontal section through a magnetic mixer,

Fig. 19 shows the magnetic mixer according to Fig. 18 in the other position, and

Fig. 20 shows the lowermost region of a cavity of a sample or reagent container.

A perspective view of a device 10 according to the invention with the preferably right-angled space coordinates x, y and z substantially comprises a central process area 12, where the separation and purifying processes take place, first guides 14, indicated only by dashed lines, for sample containers P and second guides 18 for reagent containers R. The first guides 14 in the x-direction and the second guides 18 in the y-direction have an angle α of 90°, in other words extend

at right angles. Both the samples P and the reagent containers R are substantially strip-shaped and have tubular or beaker-shaped cavities 22, which are produced by injection moulding from plastics material, the sample containers P and reagent containers R being identical. A peripheral flange 16 does not only stabilise the cavities 22, it is also used for guidance and holding.

The process area 12 is limited in its horizontal extent, largely by a carrier element 24, which consists of three carrier plates 24a, 24b and 24c. The entire carrier element 24 is lifted and lowered in the z-direction with the lowermost carrier plate 24a, and the drive and the control for this are implemented with means which are known *per se*, like the drive for supplying the sample containers P and the reagent containers R. The carrier plates 24b and 24c can be lifted and lowered together, but also individually, resulting in a relative displacement. In this case, the transfer elements 28, which are configured as permanent magnetic rods, are pushed into the membranes M or pulled out therefrom.

The carrier element 24 has a predetermined grid of holes 30, which are penetrated by permanent magnetic rods 28. These have peripheral collars, which rest on the carrier plate 24c. The membranes M are basically configured like the sample containers P and reagent containers R, but the cavities 32 are generally cylindrical. A prepared membrane M is supplied from the front left with a third guide, which is not shown for the sake of clarity. The membranes M are received at the entry to the process area 12 and conveyed during each working cycle by the grid distance a in the x-direction. This takes place by means of displacement on a horizontal pair of rails 36.

During each working cycle one filled sample container P, on the longitudinal side, reaches the process area 12. Magnetic microparticles (76 in Fig. 20) attach to the membranes M or its cavities 32 at the frontmost sample container P in the x-direction, when the permanent magnetic rods 28 are introduced. These microparticles are conveyed stepwise from reagent container R to reagent container R by way of the membranes M, with it being possible to resuspend the

attached microparticles in each working cycle. The sample containers P are ejected in the y-direction and collected in a waste container, not shown.

The reagent containers R which are supplied in the y-direction have also been filled in-line. If preassembled, filled reagent containers R are used, these are torn open or penetrated in the lid region directly before the process area 12, so a membrane M or the permanent magnetic rods 78 can be supplied. The mechanisms for filling or opening are arranged in or on a housing 38 which can also be lifted and lowered in the z-direction. In the working cycle, in the present case, six reagent containers R are introduced into the process area 12 and the used containers are ejected in the y-direction into a waste collector. Neither sample containers P nor reagent containers R are guided in the x-direction through the process area 12.

In each working cycle of about three minutes in duration, 48 samples are simultaneously separated or purified or washed. Per working cycle, eight samples leave the process area 12, in other words about 160 samples in an hour.

In Fig. 2, the process area 12 is shown at the beginning of a working cycle. The carrier plates 24a and 24b are lifted to such an extent that the membranes M are located above the level of the suspension 40 in the reagent containers R. The uppermost carrier plate 24c is lifted to such an extent that the permanent magnetic rods 28 are practically pulled out of the membranes M. The frontmost sample container P in the x-direction, the advance direction, is still outside the process area 12.

A holder 34 with a membrane M is moved to the process area 12, and the membrane M can be put over the permanent magnetic rods 28 in the z-direction. This is the first process step of a working cycle. The path of the membranes M through the entire process area 12 in the x-direction is indicated by arrows 44.

Channels 48 for the reagent containers R which are inserted from the rear, positioned in the working position according to the grid of the permanent magnetic

rods 28, and ejected to the front, which channels are open on either side, extend perpendicularly to the x-direction in the carrier block 46. Configured in an alternating manner with respect to channels 48, which are open laterally and upwardly are recesses 50, in which beams 52 which can be pushed back and forth, i.e. perpendicularly to the drawing plane, in the y-direction, are arranged, with permanent magnets, shown later in detail, for mixing the suspension 40.

A layout according to Fig. 3 shows – viewed from the top – the process area 12, in which neither sample containers P, nor reagent containers R, nor membranes M are introduced. A sample container P1 placed according to the grid in the x-direction at a spacing a is covered by a membrane M1, which is put over the permanent magnetic rods 28 from below. A buffer section 53 contains the sample containers P2 to P7, which are fed in by the filling station 54.

In the y-direction, six reagent containers R1.1 to R6.1 have been pushed at the end face to the process area 12. In this position, the reagent containers R are opened or filled with reagents. The reagent containers R of a buffer section 58 are designated by R2.1 to R6.2.

Fig. 4 shows the situation according to Fig. 3 in vertical section in the x-direction. The rearmost permanent magnetic rods 28 in the x-direction have the membrane M1 put over from below. In the x-direction, the frontmost sample container P1 is lined up at the process area 12. The latter is located precisely below the membrane M1. The holder 34 of the membrane M1 is ejected and disposed of after the membrane M1 has been lifted, and this is indicated by an arrow.

In Fig. 5, the pair of rails 36 and the carrier element 24 are completely lowered in the direction z and the membranes M1 are dipped with the inserted permanent magnetic rod 28 into the suspension of the sample container P1 placed on the longitudinal side at the process area 12. Under the action of the permanent magnetic rods 28, the magnetic microparticles of the suspension settle on the membrane M1 and remain suspended on the membrane 26 when the pair of rails 36 and the carrier element 24 are raised.

Fig. 6 shows the pair of rails 36 and carrier element 24 which have been raised to the same degree. In this position, the carrier element 24 has been displaced in the x-direction by the grid spacing a. The rearmost permanent magnetic rods 28 in the x-direction with the membrane M1 placed over are now precisely above the reagent container R.1.1 which has been introduced in the meantime. The membrane M1 has been pushed into this position according to Fig. 6 in the pair of rails 36. The reagent container 1.2, according to Fig. 7, has slipped from the buffer section 58 into the buffer section 52 where it is filled with reagents or the seal is pushed in. The reagent container R1.3 has been moved up into the buffer section 58.

Compared to the layout according to Fig. 3, the sample container P1 has been pushed away in the y-direction and falls into a waste collector, not shown. This is indicated by an arrow 60.

It is shown in Fig. 8 that the carrier element 24 with the carrier plates 24a, 24b and 24c are lowered to such an extent that the first membrane M1 has been dipped into the reagent container R1.1. Under the magnetic effect, the microparticles collect on the surface of the membrane M1. After a reaction time of about 1 minute, the carrier plate 24c with the permanent magnetic rods 28 is pulled out of the membrane M1. A magnetic mixing mechanism 62 is now started up. The magnetic microparticles are released by the mixing mechanism 62 from the membrane M1 and are resuspended.

In the next working step according to Fig. 9, the complete carrier element 24 is raised until the permanent magnetic rods 28 are completely removed from the membrane M1 which was also raised with the pair of rails 36. A second membrane M2 is then provided. The carrier element 24 can now be returned counter to the x-direction by one grid unit a and the rearmost permanent magnetic rods 28 in the x-direction are now precisely above the provided second membrane M2.

In the subsequent method step according to Fig. 10 the carrier element 24, consisting of the three carrier plates 24a, 24b and 24c, are moved down in the z-direction, until the second to rearmost permanent magnetic rods 28 in the z-direction have been dipped into the first membranes M1 in the working position. At the same time, the second membranes M2 are put over the permanent magnetic rods 28.

In Fig. 11, three process steps are combined. Firstly, the holder 34 for the second membrane M2 is removed, which is characterised by an arrow. Then, the second to frontmost sample container P2 in the x-direction is guided to the process area 12, and finally the carrier element 24 is lowered to such an extent that the first membrane M1 is dipped into the first sample container P1.1 and the second membrane M2 is dipped into the second to frontmost sample container P2 and the corresponding permanent magnetic rods 28 have been lowered into the membrane M2. The microparticles are now attached to the two first membranes M1, M2. When the permanent magnetic rods 28 are pulled up, they can be removed from the liquid phase of the suspension. The microparticle-free reagent container R1.1 can now be pushed out of the process area 12 in the y-direction and this is shown in the layout of Fig. 12. Only the first membrane M1 remains in the process area 12.

According to the following layout shown in Fig. 13, the second sample container P2 is also ejected in the y-direction and fed to the waste collector. The process containers R1.2 and R2.1 are pushed up into the process area 12 and positioned below the membranes M1 and M2.

According to Fig. 14, the carrier element 24 with the permanent magnetic rods 28 and the membranes M1 is pushed forward by a grid unit a in the x-direction and then lowered as a whole in the z-direction until the membranes M1 and M2 are positioned in the two first reagent containers R1.2 and R2.1. The magnetic microparticles are now collected on the outer wall of the membranes M1 and M2 and are attached. In this new working cycle started with Fig. 9, the procedure is now as in the previous working cycle: lifting the permanent magnetic rods 28,

mixing the releasing magnetic microparticles etc. Each new working cycle is started with the placing of a membrane M and the removal of the samples from the frontmost sample container P lined up in the x-direction, on the longitudinal side at the process area 12.

In the layout according to Fig. 15, the membrane M1 has reached the last working position in the x-direction in the process area 12. The treatment of the samples has been completed; all the provided operations have then been carried out. Only in this configuration is the device fully operable.

In the layout according to Fig. 16, it is shown that the first membrane M1, which has run through all the working cycles, is fed to the waste collector after ejection from the process area 12. The reagent container 6.1 with the result of the six working cycles, on the other hand, is supplied for use, which is indicated by the arrow 64.

Fig. 17 shows a carrier block 46, which has been broken open for the sake of clarity, of the process area 12. There are six continuous channels 48 which are open at the top, for the passage of the reagent containers R. When passing through, the reagent containers R slide with their peripheral flange 16 in opposing grooves 66 in the side walls 47 of the channels 48.

Provided between the channels 48 of the carrier block 46 are further recesses 68, which alternate with the channels 48 and are closed on the end face 70 of the carrier block 46. The recesses 68 are used for receiving beams 72, which can be pushed back and forth in the y-direction, with integrated permanent magnets 74. The permanent magnets 74 are arranged in the region of the cavities 22 of the reagent containers R and are used for mixing the resuspended magnetic microparticles 76.

As can be seen from Fig. 18 and 19, the beams 72a, 72b which can be moved back and forth are arranged on either side of the cavities 22 of a reagent container R. The permanent magnets 74 are arranged in the double spacing of

the cavities 22. When switching on, there is a relative movement between the permanent magnets 74 and the cavities 22. The microparticles 76 which are attached on one side, change side, so an effective mixing effect is produced. The effect can be improved in that the cavities are configured so as to be elliptical, oval or rectangular with round short sides.

Fig. 20 shows a greatly enlarged view of the lower region of a cavity 22 with a suspension 78. The lowermost region of a permanent magnetic rod 28 is covered with a beaker-shaped membrane M. The permanent magnets 28 have the effect that the microparticles 76 collect on the membrane M or its cavities 32. If the membrane M is removed together with the permanent magnetic rods 28, the microparticles 76 are lifted from the practically particle-free suspension 78. If, on the other hand, the permanent magnetic rod 28 is removed and the membrane M left, the microparticles 76 are released again from the membrane M. By mixing, for example as shown in Fig. 18 and 19, the release process can be accelerated and the mixing effect improved.